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## *Brief Research Communication*

# Linkage Disequilibrium Analysis of G-olf$_\alpha$ (GNAL) in Bipolar Affective Disorder

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**This study examines G-olf$_\alpha$ as a possible candidate gene for susceptibility to bipolar affective disorder (BPAD) using the Transmission Disequilibrium Test (TDT). G-olf$_\alpha$, which encodes a subunit of a G-protein involved in intracellular signaling, maps within a region of chromosome 18 that has been implicated by two different linkage studies as a potential site of BPAD susceptibility loci. The expression pattern of G-olf$_\alpha$ in the brain, its coupling to dopamine receptors, and the effects of lithium salts on G-proteins all support G-olf$_\alpha$ as a candidate gene for BPAD. Our study population consisted of 106 probands and sibs with bipolar I disorder, with a median age-at-onset of 21.5 years ascertained from the United States. There was no evidence of linkage disequilibrium between BPAD and any of the observed G-olf$_\alpha$ alleles in our sample. Division of families based on sex of the transmitting parent did not significantly change the results. This sample had good power (78%) to detect linkage disequilibrium with alleles conferring a relative risk equal to that estimated for the putative 18p locus (2.58). Our results do not support a major role for G-olf$_\alpha$ as a susceptibility locus for BPAD in a substantial portion of our sample. Other genes lying near G-olf$_\alpha$ within the linked region on chromosome 18 cannot be excluded by our data. This study illustrates the use of the TDT in evaluating candidate genes within linked chromosome regions.**


**KEY WORDS: Transmission Disequilibrium Test (TDT), G-proteins, mood disorder, genetics, bipolar I**

This study examines G-olf$_\alpha$ as a possible candidate gene for susceptibility to bipolar affective disorder (BPAD), using the Transmission Disequilibrium Test (TDT) [Spielman et al., 1993].

G-olf$_\alpha$ (a.k.a. GNAL, MIM #139312) encodes the stimulatory (alpha) subunit of a G-protein involved in intracellular signaling. G-olf$_\alpha$ has been mapped by somatic cell hybridization studies to 18p11.22–p11.21. This region has been implicated by two different linkage studies as a potential site of BPAD susceptibility loci [Berrettini et al., 1994; Stine et al., 1995]. In the study of Berrettini et al. [1994], affected sib-pair analyses indicated significant excess allele sharing for several markers in this region, although overall lod scores were not supportive of linkage. In the study of Stine et al. [1995], affected sib-pair and lod score analyses supported linkage in this same region, particularly in pedigrees with affected or transmitting fathers. These linkage results implicated G-olf$_\alpha$ as a candidate gene for BPAD.

Several other lines of evidence have also suggested G-olf$_\alpha$ as a candidate gene for BPAD. G-olf$_\alpha$ is widely expressed in the central nervous system, particularly in the basal ganglia and some limbic structures [Herve et al., 1993; Ala-Kokko et al., 1995]. G-olf$_\alpha$ is thought to be coupled to dopamine 1 receptors in the striatum and to initiate cAMP second-messenger cascades [Herve et al., 1993]. Finally, lithium salts have been implicated in altered G-protein activity in several studies [Manji et al., 1995; Carli et al., 1994].

The TDT is a test of association which does not depend on assumptions about mode of inheritance and is insensitive to population stratification [Spielman et al., 1993; Schaid and Sommer, 1994; Thomson, 1995]. The TDT compares the proportion of transmissions and nontransmissions of a marker allele to affected offspring. Since it simultaneously tests for both linkage and association, the TDT will only be positive in the presence of both true linkage and association. Thus, the TDT is less prone to false-positive results than other association methods [Lander and Schork, 1994; Thomson, 1995]. Linkage disequilibrium is usually detectable only over very short distances. The chances of detecting linkage disequilibrium over larger distances are increased when intragenic markers are used [Jorde et al.,

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1993]. Linkage disequilibrium is also more easily detected in genetically-isolated populations with strong founder effects. However, Copeman et al. [1995] successfully detected linkage disequilibrium between type 1 diabetes mellitus and markers on chromosome 2q in mixed populations, albeit more weakly than in the genetic isolate they studied. As with any association, linkage disequilibrium may be difficult to detect in a common disorder with an outbred population, especially when using a marker that does not convey altered function of the candidate gene or when there are multiple disease alleles of the same gene in the population [Crowe, 1993; Kidd, 1993].

For the present study, we used a highly-polymorphic *dinucleotide* repeat marker within the G-olf$_\alpha$ coding region. We would expect to detect linkage disequilibrium if a G-olf$_\alpha$ mutation deriving from one founder plays an important etiologic role in BPAD in our sample.

Families were ascertained through treated bipolar I (BPI) probands with 2 or more affected siblings, or one affected sibling and one and only one affected parent [for details, see Stine et al., 1995]. All families were predominantly of Northern European ancestry. For ascertainment purposes, subjects with a bipolar or unipolar disorder based on family informants were considered affected. Families with an affected phenotype among the relatives in both parental lineages, based on family informants, were excluded from study [Simpson et al., 1992].

After the procedure was explained and informed consent obtained, all subjects were interviewed by a psychiatrist trained in the use of the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) [Endicott and Spitzer, 1978]. The psychiatrists have established good interrater reliability. Best-estimate diagnoses, based on Research Diagnostic Criteria [Spitzer et al., 1975], were assigned by two noninterviewing psychiatrists.

For this study, we selected from our sample of 177 families all probands and sibs with BPI disorder (n = 106) and at least one parent for whom DNA was available. Families found to be bilineal after clinical evaluation were not excluded. Of 72 selected families, 48 contained only one affected offspring, 19 contained 2 affected offspring, 4 contained 3 affected offspring, and one family contained 6 affected offspring. The total sample size was 224 subjects. Median age-at-onset of first mania or major depression was 21.5 years. The sample was 46% male.

Genotyping procedures are detailed in Stine et al. [1995]. DNA was obtained from lymphoblastoid cell lines. A dinucleotide repeat marker (observed heterozygosity, 75%) that maps within the G-olf$_\alpha$ locus [Ala-Kokko, 1995] was amplified by PCR using standard procedures. The marker has 11 alleles, ranging in size from 106–126 base pairs (bp). The amplified products were separated on 6% denaturing polyacrylamide gel and were visualized using a Phosphorimager 425 (Molecular Dynamics, Sunnyvale, CA). Exact allele sizes in base pairs were determined using a Sequamark sequencing ladder (Research Genetics, Huntsville, AL) prepared according to the manufacturer's instructions using the Sequenase Sequencing kit version 2.0 (USB). Genotypes were rated by three independent raters blind to the diagnosis. Three genotypes could not be unambiguously identified and were excluded.

Statistical analysis was done using the Genetic Analysis System (GAS) [Young, 1995] and TDTLIKE [Terwilliger, 1995]. GAS analyzes the data using the chi-square test for each allele. TDTLIKE performs an $\alpha$-controlled analysis for multiple alleles using a likelihood approach. Observed allele frequencies were similar to those reported by Ala-Kokko [1995]. We observed 10 of the 11 reported alleles; the 108 bp allele was not found in our sample. The exact allele sizes for two heterozygous subjects as determined by our laboratory were identical to those determined by the lab of Berrettini (personal communication). The proportion of affected sib pairs sharing zero alleles identical by descent ($z_0$) was estimated using the program Mapmaker Sibs [Kruglyak and Lander, 1995].

There was no evidence of linkage disequilibrium between BPI and any of the observed G-olf$_\alpha$ alleles in our sample (Table I). The lowest *P* value in the GAS analysis was 0.24 for the 124-bp allele, with 22 instances of transmission and 14 instances of nontransmission to af-

TABLE I. Results of Linkage Disequilibrium Analyses for the G-olf$_\alpha$-Associated Marker Alleles

| Allele (bp) | Observed frequency | Transmitted | Not transmitted | *P* | |
|---|---|---|---|---|---|
| | | | | GAS[a] | TDTLIKE[b] |
| 106 | 0.066 | 6 | 6 | 1.00 | 1.00 |
| 110 | 0.001 | 0 | 0 | | |
| 112 | 0.044 | 6 | 8 | 0.79 | 1.00 |
| 114 | 0.230 | 18 | 23 | 0.53 | 1.00 |
| 116 | 0.398 | 38 | 35 | 0.82 | 0.96 |
| 118 | 0.022 | 4 | 4 | 1.00 | 1.00 |
| 120 | 0.031 | 3 | 3 | 1.00 | 1.00 |
| 122 | 0.022 | 1 | 4 | 0.37 | 1.00 |
| 124 | 0.155 | 22 | 14 | 0.24 | 0.54 |
| 126 | 0.031 | 6 | 7 | 1.00 | 1.00 |

[a]Genetic Analysis System.
[b]From Terwilliger [1995]. Maximum likelihood estimate of $\lambda$ was 0.5 ($P = 0.5$).

fected offspring. In the likelihood analysis, the lowest $P$ value was again observed for the 124-bp allele, but was not significant. Division of families based on sex of the transmitting parent did not significantly change the results. The proportions of transmitted alleles were also essentially unchanged when families with more than one affected offspring were dropped from the analysis.

Power was calculated using the formula for a matched case-control study design [Schlesselman, 1982] with a one-tailed $\alpha$-level of 0.05 and the actual transmission frequencies for the 124-bp allele. Relative risk (RR) for the power calculation was estimated from our previous linkage findings on chromosome 18p [Stine et al., 1995]. We estimate a RR of 2.58 for the 18p locus [Risch, 1990], based on the proportion of affected sib pairs sharing no alleles identical by descent ($z_0 = 0.097$) at D18S37, which is the closest linked marker to G-olf$_\alpha$ in our data set. In our sample, the power to detect linkage disequilibrium for a locus when RR = 2.58 is 78%.

This study examined G-olf$_\alpha$ as a possible candidate gene for susceptibility to BPAD using the TDT. G-olf$_\alpha$ was a good candidate gene for BPAD, since it lies within a region that has been linked to BPAD, and since it encodes a G-protein involved in signal transduction. However, our results do not support a major role for G-olf$_\alpha$ as a susceptibility locus for BPAD in a substantial portion of our sample. Although we cannot exclude a role for G-olf$_\alpha$ in some cases of BPAD, this sample had good power to detect an association with alleles conferring RR equal to that estimated for the putative 18p locus. Other genes lying near G-olf$_\alpha$ within the linked region on chromosome 18 cannot be excluded by our data.

Linkage disequilibrium analysis has great promise as a tool in the genetic study of complex phenotypes. Linkage disequilibrium analysis by the TDT avoids variables that are usually uncertain in linkage and association studies, such as mode of inheritance and population stratification [Thomson, 1995]. Thus, the TDT often gives simpler, less ambiguous results than traditional approaches. Linkage disequilibrium may be easily detected over only very small genetic distances or in genetically isolated populations with strong founder effects, limiting its application as a screening tool in most populations [Jorde, 1995]. However, markers that lie within candidate genes usually display greater linkage disequilibrium [Jorde et al., 1993], and may provide data to help implicate a candidate gene or exclude it from further consideration.

This study illustrates the use of the TDT in evaluating candidate genes within linked chromosome regions. Our results do not indicate linkage disequilibrium between BPAD and alleles of G-olf$_\alpha$ in our study population. Future studies may benefit from applying this approach to other populations, to other candidate genes on chromosome 18, or throughout the genome.

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